At page 39, please replace the paragraph beginning at line 16 with the following:

A12

-- Fragment F2 and the dephosphorylated plasmid V2 were ligated with T4 DNA ligase. E.coli HB101 cells were then transformed and bacteria identified that contained the plasmid (pBacPTH receptor) with the PTH receptor gene using the enzymes SmaI and XbaI. The sequence of the cloned fragment was confirmed by DNA sequencing.--

In the Drawings

Please replace the informal drawings of Figures 1-3 with the formal drawings of Figures 1A-1E, 2A-2B, and 3A-3C.

REMARKS

The specification has been amended to claim benefit of priority of the related applications, to update the address of the ATCC, to properly identify the Sequence Listing SEQ ID numbers for sequences shown in Figure 3A-3C to correct minor typographical errors, and to enter the Substitute Sequence Listing. The drawings have been amended to replace informal drawings of Figures 1-3 with formal drawings of Figures 1A-1C, 2A-2B, and 3A-3C of the instant application.

Statements Under 37 C.F.R. § 1.825(a) and (b)

Applicants herewith submit a paper copy of a Substitute Sequence Listing (12 pages) and, a computer-readable diskette containing the Substitute Sequence Listing. The Substitute Sequence Listing (paper copy and computer readable form (CRF)) is submitted herewith to comply with current format requirements under 37 C.F.R. § 1.823 and § 1.824.

As such, in accordance with 37 C.F.R. § 1.825(a), the undersigned attorney for Applicants hereby states that sequence information contained in the Substitute Sequence Listing submitted herewith is identical to the sequence information filed with original Application No. 08/468,011. The Substitute Sequence listing is completely supported by the specification as originally filed, and no new matter has been introduced.

In accordance with 37 C.F.R. § 1.825(b), the undersigned attorney for Applicants hereby states that the information in the paper copy of the Substitute Sequence Listing submitted herewith is identical to the information contained in the computer readable form of the Substitute Sequence Listing submitted herewith.

No new matter has been added by these amendments.

Conclusion

Entry and consideration of the above amendments and remarks are respectfully requested.

Applicants respectfully request entry of the amendments and remarks contained in this paper. It is believed that no charge is required for this Amendment. In the event a fee is required, please charge the required fee to Deposit Account No. 08-3425.

Respectfully submitted,

Dated: NOVEMBER 30, 2001

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Enclosures
KKH/JMM/DS/lcc/kp

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: SOPPET, et al.

Application Serial No.: To be assigned

Art Unit: To be assigned

Filed: Concurrently herewith

Examiner: To be assigned

For: G-Protein Parathyroid Hormone Receptor HLTDG74

Atty. Docket No.: PF201D2

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Text has been amended as indicated by brackets [ ] (for deletions) and underlining (for additions).

In the Specification:

Page 1, immediately after the title, a new paragraph claiming priority to related applications has been added.

At page 6, line 9, immediately before the paragraph which begins "The following drawings..." the following subheading has been added:

-- Brief Description of the Drawings --

At page 6, the paragraph beginning at line 12 has been amended as follows:

Figure 1 shows the cDNA sequence (SEQ ID NO:1) and the corresponding deduced amino acid sequence (SEQ ID NO:2) of the G-protein PTH receptor of the present invention. The standard one-letter abbreviation for amino acids is used. Sequencing was performed using a 373 Automated DNA sequencer (Applied Biosystems, Inc.).

At page 7, the paragraph beginning at line 3 has been amended as follows:

Figure 3 illustrates an amino acid alignment of the G-protein PTH receptor of the present invention (top line) and the human PTH receptor (bottom line). In Figure 3, the "Query:" line refers to the polypeptide sequence portion of the polypeptide according to the invention and the "Sbjct." line refers to the comparative portions from human PHT receptor protein. Further, in Figure 3 the polypeptide segments set forth in Query:729-908/Sbjct.:253-312; Query:909-1088/Sbjct.:313-372; Query:1089-1244/Sbjct.:373-424; Query:267-446/Sbjct.:102-161; Query:447-476/Sbjct.:162-171; Query:498-677/Sbjct.:177-236; Query:678-740/Sbjct.:237-257; Query:1248-1424/Sbjct.:427-485; Query:159-269/Sbjct.:24-60; and Query:1508-1576/Sbjct.:512-534 correspond to SEQ ID NOS:9-28, respectively.

At page 7, the paragraph beginning at line 7 has been amended as follows:

In accordance with an aspect of the present invention, there is provided an isolated nucleic acid (polynucleotide) which encodes for the mature polypeptide having the deduced amino acid sequence of Figure 1 (SEQ ID NO:2) or for the mature polypeptide encoded by the cDNA of the clone deposited as ATCC Deposit No. [____ on June 2, 1995] 97186 on June 1, 1995 with the American Type Culture Collection (ATCC) at 10801 University Boulevard, Manassas, VA 20110-2209 under terms of the Budapest Treaty.

At page 34, the paragraph beginning at line 32 and continuing to page 36 has been amended as follows:

-- The DNA sequence encoding for PTH receptor, ATCC [# ___] No. 97186 is initially amplified using PCR oligonucleotide primers corresponding to the 5' and sequences of the processed protein and the vector sequences 3' to the PTH receptor gene. Additional nucleotides corresponding to the PTH receptor were added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence CAGCCGTCCCGGGCTTGGCCTGG (SEQ ID NO:3) contains a [SMaI] SmaI restriction enzyme site followed by 6 nucleotides of the PTH receptor coding sequence starting from the presumed second amino acid of the processed protein codon. The 3' sequence CCTCAGTGTCGACTTGTCATCCTTCAG (SEQ ID NO:4) contains complementary sequences to [SALI] Sal I site and is followed by 6 nucleotides encoding the PTH receptor.

The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector PQE-30. (Qiagen, Inc. Chatsworth, CA, 91311). PQE-30 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. PQE-30 was then digested with [SMAI] SmaI and [SALI] Sal I. The amplified sequences were ligated into PQE-30 and were inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture was then used to transform E. coli available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by centrifugation. The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized PTH receptor was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). The PTH receptor was eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate. --

At page 37, the paragraph beginning at line 3 has been amended as follows:

-- The DNA sequence encoding for PTH receptor, ATCC [# ____] No. 97186, was constructed by PCR using two primers: the 5' primer (GTTGGCATATTGGAAGCTTTTTGCGGG) (SEQ ID NO:5) contains a [HINDIII] Hind III site 5 UTR; the 3' sequence (CAGTTTCTAGATGTCATCCTTCAGTGTC (SEQ ID NO:6) contains complementary sequences to XbaI site, translation stop codon, and the last 12 nucleotides of the PTH receptor coding sequence (not including the stop codon). Therefore, the PCR product contains a [HINDIII] Hind III site, PTH receptor coding sequence followed by a translation termination stop codon and an XbaI site. The PCR amplified DNA fragment and the vector, pcDNA3/Amp, were digested with [HINDIII] Hind III and XbaI restriction enzyme and ligated. The ligation mixture was transformed into E. coli strain [DA5α] DH5α (available from Stratagene Cloning Systems, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant PTH receptor, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the PTH receptor HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5). (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels. --

At page 38, the paragraph beginning at line 4 has been amended as follows:

The DNA sequence encoding the full length PTH receptor protein, ATCC [# ___] No. 97186, was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

At page 38, the paragraph beginning at line 8 has been amended as follows:

The 5' primer has the sequence TCCTACCCGGGCCGCCATCATGGC CTGGCTGGGGGCCT (SEQ ID NO:7) and contains a [SMAI] SmaI restriction enzyme site (in bold) followed by 8 nucleotides resembling an efficient signal for the initiation of translation in eukaryotic cells (J. Mol. Biol. 1987, 196, 947-950, Kozak, M.), and just behind the first 19 nucleotides of the PTH receptor gene (the initiation codon for translation "ATG" is underlined).

At page 38, the paragraph beginning at line 16 has been amended as follows:

The 3' primer has the sequence CAGTTTCTAGATGTCATCCTTCAGTGTC (SEQ ID NO:8) and contains the cleavage site for the restriction endonuclease XbaI and 13 nucleotides complementary to the 3' non-translated sequence of the PTH receptor gene. The amplified sequences were isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment was then digested with the endonucleases [SMAI] SmaI and XbaI and then purified. This fragment is designated F2.

At page 38, the paragraph beginning at line 25 has been amended as follows:

The vector pRG1 (modification of pVL941 vector, discussed below) is used for the expression of the PTH receptor protein using the baculovirus expression system (for review see: Summers, M.D. and Smith, G.E. 1987, A manual of methods for baculovirus vectors and insect cell culture procedures, Texas Agricultural Experimental Station Bulletin No. 1555). This expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by the recognition sites for the restriction endonucleases [SMAI] SmaI and XbaI. The polyadenylation site of the simian virus (SV)40 is used for efficient polyadenylation. For an easy selection of recombinant viruses the beta-galactosidase gene from E.coli is inserted in the same orientation as the polyhedrin promoter followed by the polyadenylation signal of the polyhedrin gene. The polyhedrin sequences are flanked at both sides by viral sequences for the cell-mediated homologous recombination of co-transfected wild-type viral DNA.

Many other baculovirus vectors could be used in place of pRG1 such as pAc373, pVL941 and pAcIM1 (Luckow, V.A. and Summers, M.D., Virology, 170:31-39).

At page 39, the paragraph beginning at line 11 has been amended as follows:

The plasmid was digested with the restriction enzymes [SMAI] SmaI and XbaI and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The DNA was then isolated from a 1% agarose gel as described in Example 1. This vector DNA is designated V2.

At page 39, the paragraph beginning at line 16 has been amended as follows:

Fragment F2 and the dephosphorylated plasmid V2 were ligated with T4 DNA ligase. E.coli HB101 cells were then transformed and bacteria identified that contained the plasmid (pBacPTH receptor) with the PTH receptor gene using the enzymes [SMAI] SmaI and XbaI. The sequence of the cloned fragment was confirmed by DNA sequencing.